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(54) Title: NUCLEIC ACID, POLYPEPTIDES, ASSAYS, THERAPEUTIC METHODS AND MEANS

(57) Abstract: Human protein termed herein B7-3 encoded by the B7-3 gene has been cloned and characterised and can be made recombinantly and used. B7-3 protein is a ligand for inducible co-stimulator protein (ICOS). A soluble form of B7-3, for example comprising the extracellular domain shown to bind ICOS, is further provided, as are assay methods for obtaining agents for modulation of the interaction between B7-3 and ICOS.

NUCLEIC ACID, POLYPEPTIDES, ASSAYS,  
THERAPEUTIC METHODS AND MEANS

The present invention relates to a novel human protein termed  
5 herein B7-3 encoded by the B7-3 gene, cloned and  
characterised by the inventor. It relates to methods of  
production and use of the protein, variants and fragments  
thereof, such as a soluble form (e.g. comprising the  
extracellular domain shown to bind ICOS). B7-3 is identified  
10 as a ligand for ICOS ("inducible co-stimulator" - Hutloff et  
al. (1999) *Nature* 397: 263-266) and further aspects of the  
invention provide for modulation of the interaction between  
B7-3 and ICOS.

15 The course of immune responses is highly dependent on the  
interactions of leukocyte cell surface molecules. The most  
important of these include the interactions of the B7  
molecules, B7-1(CD80) and B7-2(CD86) expressed on antigen  
presenting cells (APCs), which bind to CD28/CTLA-4(CD152)  
20 expressed on T cells.

In numerous studies of experimental disease models, the  
blockade of interactions involving the B7-like molecules has  
been shown to have profound effects. For example, the  
25 transfection of B7-1 into murine tumours has lead to their  
regression and on occasion given protection to subsequent  
challenges with parental tumours lacking B7-1 expression

(Chen et al. (1992) Cell 71, 1093-1102; Townsend and Allison (1993) Science 259, 368-370). Similarly, enhanced tumour immunity has been obtained by blocking CTLA-4 engagement with anti-CTLA-4 antibodies (Leach et al. (1996) Science 271, 1734-1736). In a different therapeutic setting, the blockade of B7-CD28 interactions using chimeric constructs consisting of CTLA-4 and immunoglobulin Fc affect general immunosuppression (Linsley et al. (1992) Science 257, 792-795) and, in combination with the blockade of the CD40 signalling pathway, enhances skin and cardiac allograft survival (Larsen et al. (1996) Nature 381, 434-438). The blockade of B7/CD28 interactions reduces autoantibody production and morbidity in a murine lupus model (Finck et al. (1994) Science 265, 1225-1227). These studies have stimulated considerable interest in the therapeutic potential of manipulating these types of interactions in the clinic.

ICOS ("inducible co-stimulator" - Hutloff et al. (1999) Nature 397, 263-266), is structurally and functionally related to CD28 and expressed on T cells

The present invention is based on identification of a ligand for ICOS and cloning of encoding nucleic acid. The ligand is termed B7-3 and the encoding gene *B7-3*.

25

B7-3 corresponds to previously anonymous database entries. Work published by Henry et al. (Immunogenetics (1997) 46,

383-95) provided a suggestion that several new, largely uncharacterised B7-related proteins existed. Two of these, whose binding specificities had not been determined, were referred to as B7c and TU-D (transcription unit D) by Henry  
5 et al.

The sequence referred to as B7c by Henry et al. was full length, but the sequence they referred to as TU-D was an incomplete EST. The present inventor performed database  
10 searching with the TU-D EST and identified an uncharacterised cDNA which was deposited in the DDBJ/EMBL/GenBank databases on 06-FEB-1999 and was derived from a human brain cDNA library. This gene was referred to as KIAA0653 protein by its Japanese depositors (accession number AB014553), who believed  
15 the sequence to be a partial gene sequence.

The present inventor determined that the gene deposited as KIAA0653 and indicated to be partial in fact encoded the full length TU-D sequence.

20

As described in detail below, the inventor experimentally determined that the encoded protein (renamed B7-3) is a ligand of ICOS, giving rise to various aspects of the present invention.

25

A chicken B7-like gene was previously identified by O'Regan et al. (Immunogenetics (1999) 49, 68-71) by expression cloning using CTLA-4 as a panning ligand.

In various aspects the present invention provides encoding nucleic acid, recombinant vectors and host cells, the encoded B7-3 polypeptide, methods of making the polypeptide by expression from the encoding nucleic acid, and so on.

5

Furthermore, the disclosure provides functional information for the protein and the invention further provides means, assays, and methods for the development of novel diagnostic, prophylactic and therapeutic agents for diseases such as *H.*

10 *pylori* induced peptic ulcers, Crohn's disease, multiple sclerosis, type 1 diabetes mellitus, graft rejection, helminth infections, allergic diseases and other disease states that may be associated with aberrant function of this protein. Of particular interest is modulation of interaction  
15 between B7-3 and ICOS in order to affect IL-10 production, T cell response to antigen (e.g. proliferation, secretion or lymphokines, upregulation of molecules that mediate cell-cell interaction, and/or help for antibody secretion by B cells) and/or TH1/TH2 bias in the immune system. Assays and screens  
20 for substances able to modulate such interaction are provided as aspects of the present invention.

ICOS is a protein of Mr 55,000-60,000 expressed upon activation of human T cells (Hutloff et al.). With similar  
25 potency to that of CD28, ICOS stimulates the key responses to foreign antigens, including proliferation, lymphokine secretion, T helper function and upregulation of several of

the molecules implicated in the interactions of leukocytes. In contrast to CD28, however, ICOS induces the secretion of IL-10 rather than IL-2. This provides indication that the natural interactions of ICOS with its ligand(s) (B7-3) might potentially favour T cell differentiation along the TH2 pathway, simultaneously disfavouring the TH1 pathway differentiation.

Accordingly, interference of the interactions of ICOS with B7-3 may be used to inhibit or block production of IL-10 (e.g. with antibodies to B7-3), or to induce ICOS signal transduction and IL-10 production (e.g. with tetrameric forms of B7-3), indicated to be of therapeutic benefit in the context of TH2 cell- and TH1-cell dependent human diseases.

15

Upon activation, T cells can differentiate into one of two different populations (reviewed by Mosmann and Sad (1996) Immunol Today 17, 138-146). The two populations are characterised by different patterns of cytokine secretion and distinct effector functions. TH1 cells produce IL-2, IFN $\gamma$  and TNF- $\beta$  which elicit macrophage activation and induce delayed-type hypersensitivity reactions, whereas TH2 cells secrete IL-4, IL-5, IL-10 and IL-13 which act as growth factors for mast cells, eosinophils and B cells and which inhibit macrophage functions. The cross-regulatory effects of some of these cytokines have been shown in some circumstances to lead to polarised TH1 and TH2 responses. These types of responses have in turn been implicated in a variety of human autoimmune

and infectious diseases, and in transplant rejection  
(reviewed by D'Elios and Del Prete (1998) Trans Proc 30,  
2373-2377). Examples of human diseases for which particular T  
cell populations have been attributed pathological roles, and  
5 in which modulation of B7-3/ICOS interaction in accordance  
with the present invention may be beneficial, include *H.*  
*pylori* induced peptic ulcers, Crohn's disease, multiple  
sclerosis, type 1 diabetes mellitus and graft rejection for  
TH1 cells, and helminth infections and a variety of allergic  
10 diseases for TH2 cells (reviewed by D'Elios and Del Prete  
(1998) Trans Proc 30, 2373-2377). Altering the balance of  
cytokine production by blocking particular cell-cell  
interactions or by inducing signalling by particular  
receptors may be used in amelioration of these conditions.

15

Various aspects of the present invention provide for the use  
of B7-3 or B7-3 and ICOS in screening methods and assays for  
agents which modulate B7-3 function and/or interaction  
between B7-3 and ICOS.

20

Such molecules may be identified by various means. For  
instance, information may be obtained about residues which  
are important for B7-3/ICOS interaction using alanine  
scanning and deletion analysis of B7-3 and/or ICOS, and/or  
25 peptide fragments of either. When key residues are  
identified, computer sequence databases may be scanned for  
proteins including the same or similar pattern of residues,



taking into account conservative variation in sequence (see below) as appropriate. Candidate molecules may then be used in one or more assays for interaction with B7-3.

- 5 Knowledge of B7-3/ICOS interaction may also be used in the design of peptide and non-peptidyl agents which modulate, particularly inhibit, such interaction as discussed further below.
- 10 Methods of obtaining agents able to modulate B7-3 function and/or interaction between B7-3 and ICOS include methods wherein a suitable end-point is used to assess interaction in the presence and absence of a test substance. Assay systems may be used to determine activity of a B7-3- or ICOS-
- 15 dependent pathway, for instance IL-10 production and/or TH2 activity.

B7-3 and its interaction with ICOS provides a novel therapeutic target, e.g. via assays including scintillation

- 20 proximity assays, direct protein-protein interactions *in vitro*, peptide-protein interactions *in vitro*, and so on.

Mimicking this reaction may be used in stimulating IL-10 production by T-cells and/or a bias to TH2 T-cell activity. Conversely, inhibiting or perturbing this interaction may be

- 25 used to prevent cells functioning in the appropriate way, and may inhibit IL-10 production and/or provide a bias away from TH2 T-cell activity towards TH1. As noted, modulating B7-3

interaction with ICOS may be used to affect processes involved in disorders involving inappropriate TH1 or TH2 activity (e.g. *H. pylori* induced peptic ulcers, Crohn's disease, multiple sclerosis, type 1 diabetes mellitus, graft rejection, helminth infections and allergic diseases).

Given the results and rationalisation reported herein on which the present invention is based, activators and inhibitors of B7-3 activity may be identified and appropriate agents may be obtained, designed and used for modulating TH1/TH2 bias, IL-10 production, T cell response to antigen (e.g. proliferation, secretion or lymphokines, upregulation of molecules that mediate cell-cell interaction, and/or help for antibody secretion by B cells) and treatment of a disorder involving any of these.

An assay according to the present invention as discussed further below may determine the role of B7-3 on any of these interactions and an agent found to be able to modulate such interaction may be used to disrupt or promote any of these interactions, e.g. in a therapeutic context.

Interaction between B7-3 and ICOS or other component may be inhibited by inhibition of the production of the relevant protein. For instance, production of one or more of these components may be inhibited by using appropriate nucleic acid to influence expression by antisense regulation. The use of anti-sense genes or partial gene sequences to down-regulate

gene expression is now well-established. Double-stranded DNA is placed under the control of a promoter in a "reverse orientation" such that transcription of the "anti-sense" strand of the DNA yields RNA which is complementary to normal mRNA transcribed from the "sense" strand of the target gene. The complementary anti-sense RNA sequence is thought then to bind with mRNA to form a duplex, inhibiting translation of the endogenous mRNA from the target gene into protein. Whether or not this is the actual mode of action is still uncertain. However, it is established fact that the technique works.

Another possibility is that nucleic acid is used which on transcription produces a ribozyme, able to cut nucleic acid at a specific site - thus also useful in influencing gene expression. Background references for ribozymes include Kashani-Sabet and Scanlon (1995). *Cancer Gene Therapy*, 2, (3) 213-223, and Mercola and Cohen (1995). *Cancer Gene Therapy* 2, (1) 47-59.

20

Thus, various methods and uses of modulators, which modulate (inhibit or potentiate) B7-3 function and/or inhibit or potentiate interaction of B7-3 and ICOS are provided as further aspects of the present invention. The purpose of disruption, interference with or modulation of interaction between B7-3 and ICOS may be to modulate any activity mediated by virtue of such interaction, as discussed above

and further below.

B7-3 may function in concert with one or more additional peptides or polypeptides in addition to ICOS. The present invention provides assays to detect these, for example making  
5 use of antibodies such as provided herein (or equivalents, or antisera or antibodies or monoclonal antibodies to different parts of the protein). Antibodies or other means available in the art may be used to study the purification of B7-3 from  
10 mammalian cell extracts. This may involve purifying or part-purifying to part or full purity and looking for associated factors, which may be cloned using protein micro-sequencing etc. Interacting molecules may be identified using, for example, a two-hybrid screen. Such other factors and/or  
15 their interactions with B7-3 may be used to derive additional inhibitors/activators of B7-3-dependent signalling pathways.

Assays according to the present invention may be used in the identification of such additional polypeptides, for example  
20 by assaying for protein fractions that stimulate IL-10 production or TH2 activity. The present invention also provides for the use of B7-3 in identifying and/or obtaining such factors.

25 Protein or other co-factors of B7-3 or ICOS may be used in the design of inhibitors, providing another route for modulating the activity of interest. This may similarly be used to provide a route to deriving agents that activate

ICOS-mediated activity.

According to a first aspect of the present invention there is provided nucleic acid consisting essentially of a nucleotide  
5 sequence encoding the amino acid sequence shown in SEQ ID NO. 2 for B7-3.

"B7-3 nucleic acid" in accordance with the present invention includes a nucleic acid molecule which has a nucleotide  
10 sequence encoding a polypeptide which includes the amino acid sequence shown in SEQ ID NO. 2.

The term "B7-3 gene" or "B7-3 allele" includes normal alleles of the B7-3 gene, and also alleles carrying one or more  
15 variations that are linked to a predisposition to a disorder as explained below. Alleles including such mutations are also known in the art as susceptibility alleles.

The coding sequence may be that shown in SEQ ID NO. 1 or it  
20 may be a mutant, variant, derivative or allele thereof. The sequence may differ from that shown by a change which is one or more of addition, insertion, deletion and substitution of one or more nucleotides of the sequence shown. Changes to a nucleotide sequence may result in an amino acid change at the  
25 protein level, or not, as determined by the genetic code. Thus, nucleic acid according to the present invention may include a sequence different from the sequence shown in SEQ

ID NO. 1 yet encode a polypeptide with the same amino acid sequence.

On the other hand, the encoded polypeptide may comprise an  
5 amino acid sequence which differs by one or more amino acid  
residues from the amino acid sequence shown in SEQ ID NO. 2.  
Nucleic acid encoding a polypeptide which is an amino acid  
sequence mutant, variant, derivative or allele of the  
sequence shown in SEQ ID NO. 2 is further provided by the  
10 present invention. Such polypeptides are discussed below.

Mutants, alleles, variants and derivatives of the specific  
sequences provided herein may have homology to the specific  
sequences in any of the terms described below in relation to  
15 use of peptides or polypeptides in different assays and other  
methods of the invention.

Methods for production of a polypeptide or peptide from  
encoding nucleic acid, production and use of suitable host  
20 cells, and other manipulations of nucleic acid are also  
discussed further below.

The present invention in various aspects also provides for  
modulating, interfering with or interrupting, increasing or  
25 potentiating B7-3 activity and/or B7-3 interaction with ICOS,  
using an appropriate agent.

The amino acid and nucleic acid sequences for ICOS are

provided by Hutloff et al. (1999) *Nature* 397: 263-266 and have also been deposited (on 1 February 1999) as "activation-inducible lymphocyte immunomediatory molecule" (AILIM) by Tezuka et al. in the DDBJ/EMBL/GenBank databases under  
5 accession number AB023135.

Agents useful in accordance with the present invention may be identified by screening techniques which involve determining whether an agent under test binds or interacts with B7-3,  
10 and/or inhibits or disrupts the interaction of B7-3 protein or a suitable fragment thereof (e.g. extracellular domain such as contained within SEQUENCE ID NO. 4) with ICOS or a fragment thereof, or a suitable analogue, fragment or variant thereof.

15

Suitable fragments of B7-3 or ICOS include those which include residues which interact with the counterpart protein. Smaller fragments, and analogues and variants of this fragment may similarly be employed, e.g. as identified using  
20 techniques such as deletion analysis or alanine scanning.

By analogy with B7-1, the ligand binding face can be predicted to consist of the GFCC'C"  $\beta$ -sheet of B7-3. Using the ClustalW program (CLUSTALW (1.74) MULTIPLE SEQUENCE  
25 ALIGNMENTS, Higgins et al, *Methods Enzymology*, (1996) 266 383-402, employing default parameters) to align the sequence of B7-3 with that of B7-1,  $\beta$ -strand sequences useful in

design of inhibitors of the interaction of B7-3 with ICOS  
include:

	$\beta$ -strand C:	DVYVYWQT	(residues 49-56)
5	$\beta$ -strand C':	KTVVTYHI	(residues 60-67)
	$\beta$ -strand C":	LENVD	(residues 73-77)
	$\beta$ -strand F:	QKFHCLVLSQ	(residues 109-118)
	$\beta$ -strand G:	GFQEVLSVEVTLHV	(residues 121-134)

10 (Residue numbering refers to the pre-protein sequence of  
SEQUENCE ID NO. 2)

Thus, the present invention provides a peptide fragment of  
B7-3 which is able to interact with ICOS and/or inhibit  
15 interaction between B7-3 and ICOS, and provides a peptide  
fragment of ICOS which is able to interact with B7-3 and/or  
inhibit interaction between ICOS and B7-3, such peptide  
fragments being obtainable by means of deletion analysis  
and/or alanine scanning of the relevant protein - making an  
20 appropriate mutation in sequence, bringing together a mutated  
fragment of one of the proteins with the other or a fragment  
thereof and determining interaction. In preferred  
embodiments, the peptide is short, as discussed below, and  
may be a minimal portion that is able to interact with the  
25 relevant counterpart protein and/or inhibit the relevant  
interaction.



Preferred peptides according to embodiments of the present invention consist of or comprise one or more of the peptide sequences given above.

- 5 Screening methods and assays are discussed in further detail below.

One class of agents that can be used to affect B7-3 activity and/or disrupt the interaction of B7-3 and ICOS are peptides  
10 based on the sequence motifs of B7-3 or ICOS that interact with counterpart ICOS or B7-3 (as discussed already above). Such peptides tend to be short, and may be about 40 amino acids in length or less, preferably about 35 amino acids in length or less, more preferably about 30 amino acids in  
15 length, or less, more preferably about 25 amino acids or less, more preferably about 20 amino acids or less, more preferably about 15 amino acids or less, more preferably about 10 amino acids or less, or 9, 8, 7, 6, 5 or less in length. Peptides of the invention may be about 10-20 amino  
20 acids, about 10-30 amino acids, about 20-30 amino acids or about 30-40 amino acids in length. The present invention also encompasses peptides which are sequence variants or derivatives of a wild type B7-3 or ICOS sequence, but which retain ability to interact with ICOS or B7-3 (respectively,  
25 as the case may be) and/or ability to modulate interaction between B7-3 and ICOS.

A further aspect of the invention provides B7-3 or a B7-3

fragment (e.g. extracellular domain - SEQUENCE ID NO. 4, preferably with signal sequence removed) in a tetrameric form. Tetrameric B7-3 may be used to stimulate ICOS and so, for example, stimulate IL-10 production and/or a shift  
5 towards TH2 activity in the immune system (with therapeutic and other applications as discussed). Tetramerisation may employ biotinylation with use of avidin or streptavidin. An example of a procedure for chemically biotinylating proteins for the purpose of generating tetrameric cell surface  
10 molecules is given in Altman et al (1996) Science, 274: 94-96. O'Callaghan et al (1999) Anal Biochem 266: 9-15 is an example of a description of a method for enzymatically biotinylating a protein.

15 A further aspect of the invention provides a method comprising biotinylating a B7-3 polypeptide or fragment as disclosed and providing avidin or streptavidin to form a B7-3 tetramer. Biotinylation and tetramerisation may follow production of the B7-3 polypeptide by expression from  
20 encoding nucleic acid.

Still further aspects provide the use of B7-3 in the production of a B7-3 tetramer, and the use of a B7-3 tetramer in binding to ICOS and/or stimulating ICOS, e.g. stimulating  
25 IL-10 production and/or TH2 activity of the immune system.

Another aspect of the present invention provides a method of stimulating ICOS activity (e.g. stimulating IL-10 production

and/or TH2 activity of the immune system), the method comprising contacting ICOS on T-cells with a B7-3 tetramer as disclosed.

- 5 As noted, other aspects of the present invention provide methods of inhibiting ICOS activity (e.g. inhibiting IL-10 production and/or TH2 activity of the immune system), such methods comprising contacting ICOS on T-cells with a B7-3 fragment, an antibody, peptide, mimetic or other substance  
10 which inhibits interaction between B7-3 and ICOS.

Instead of using a wild-type B7-3 or wild-type B7-3 and/or ICOS fragment, a peptide or polypeptide may include an amino acid sequence which differs by one or more amino acid  
15 residues from the wild-type amino acid sequence, by one or more of addition, insertion, deletion and substitution of one or more amino acids. Thus, variants, derivatives, alleles and mutants are included.

- 20 Preferably, the amino acid sequence shares homology with a fragment of the relevant B7-3 or ICOS fragment sequence, preferably at least about 30%, or 40%, or 50%, or 60%, or 70%, or 75%, or 80%, or 85%, 90% or 95% homology. Thus, a peptide fragment of B7-3 or ICOS may include 1, 2, 3, 4, 5,  
25 greater than 5, or greater than 10 amino acid alterations such as substitutions with respect to the wild-type sequence.

A derivative of a peptide for which the specific sequence is

disclosed herein may be in certain embodiments the same length or shorter than the specific peptide. In other embodiments the peptide sequence or a variant thereof may be included in a larger peptide, as discussed above, which may or may not include an additional portion of B7-3 or ICOS. 1, 2, 3, 4 or 5 or more additional amino acids, adjacent to the relevant specific peptide fragment in B7-3 or ICOS, or heterologous thereto may be included at one end or both ends of the peptide.

10

As is well-understood, homology at the amino acid level is generally in terms of amino acid similarity or identity. Similarity allows for "conservative variation", i.e. substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine.

20 Similarity may be as defined and determined by the TBLASTN or other BLAST program, of Altschul et al., (1990) *J. Mol. Biol.* 215, 403-10, which is in standard use in the art, or, and this may be preferred, either of the standard programs BestFit and GAP, which are part of the Wisconsin Package, Version 8, September 1994, (Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA, Wisconsin 53711). BestFit makes an optimal alignment of the best segment of

similarity between two sequences. Optimal alignments are found by inserting gaps to maximize the number of matches using the local homology algorithm of Smith and Waterman (Advances in Applied Mathematics (1981) 2, pp. 482-489). GAP  
5 uses the Needleman and Wunsch algorithm to align two complete sequences that maximizes the number of matches and minimizes the number of gaps. Generally, the default parameters are used, with a gap creation penalty = 12 and gap extension penalty = 4.

10

Homology may be over the full-length of the relevant peptide or over a contiguous sequence of about 5, 10, 15, 20, 25, 30, 35, 50, 75, 100 or more amino acids, compared with the relevant wild-type amino acid sequence.

15

Homologous nucleic acid may be identified or its presence confirmed by means of hybridization experiments, for instance involving Southern blotting.

20 For example, hybridizations may be performed, according to the method of Sambrook et al. (below) using a hybridization solution comprising: 5X SSC (wherein 'SSC' = 0.15 M sodium chloride; 0.15 M sodium citrate; pH 7), 5X Denhardt's reagent, 0.5-1.0% SDS, 100 µg/ml denatured, fragmented salmon  
25 sperm DNA, 0.05% sodium pyrophosphate and up to 50% formamide. Hybridization is carried out at 37-42°C for at least six hours. Following hybridization, filters are washed as follows: (1) 5 minutes at room temperature in 2X SSC and

1% SDS; (2) 15 minutes at room temperature in 2X SSC and 0.1% SDS; (3) 30 minutes - 1 hour at 37°C in 1X SSC and 1% SDS; (4) 2 hours at 42-65°C in 1X SSC and 1% SDS, changing the solution every 30 minutes.

5

One common formula for calculating the stringency conditions required to achieve hybridization between nucleic acid molecules of a specified sequence homology is (Sambrook et al., 1989):  $T_m = 81.5^{\circ}\text{C} + 16.6\text{Log} [\text{Na}^+] + 0.41 (\% \text{ G+C}) - 0.63$   
10  $(\% \text{ formamide}) - 600/\text{\#bp in duplex}.$

As an illustration of the above formula, using  $[\text{Na}^+] = [0.368]$  and 50-% formamide, with GC content of 42% and an average probe size of 200 bases, the  $T_m$  is 57°C. The  $T_m$  of a  
15 DNA duplex decreases by 1 - 1.5°C with every 1% decrease in homology. Thus, targets with greater than about 75% sequence identity would be observed using a hybridization temperature of 42°C. Such a sequence would be considered substantially homologous to the nucleic acid sequence of the present  
20 invention.

It is well known in the art to increase stringency of hybridisation gradually until only a few positive clones remain. Other suitable conditions include, e.g. for detection  
25 of sequences that are about 80-90% identical, hybridization overnight at 42°C in 0.25M  $\text{Na}_2\text{HPO}_4$ , pH 7.2, 6.5% SDS, 10% dextran sulfate and a final wash at 55°C in 0.1X SSC, 0.1% SDS. For detection of sequences that are greater than about

90% identical, suitable conditions include hybridization overnight at 65°C in 0.25M Na<sub>2</sub>HPO<sub>4</sub>, pH 7.2, 6.5% SDS, 10% dextran sulfate and a final wash at 60°C in 0.1X SSC, 0.1% SDS.

5

As noted, variant peptide sequences and peptide and non-peptide analogues and mimetics may be employed, as discussed further below.

- 10 Various aspects of the present invention provide a substance, which may be a single molecule or a composition including two or more components, which includes a peptide fragment of B7-3, a peptide consisting essentially of such a sequence, a peptide including a variant, derivative or analogue sequence,
- 15 or a non-peptide analogue or mimetic which has the ability to modulate B7-3 activity, and/or interact with B7-3 or ICOS and/or modulate, disrupt or interfere with interaction between B7-3 and ICOS, directly or indirectly via one or more factors.

20

Variants include peptides in which individual amino acids can be substituted by other amino acids which are closely related as is understood in the art and indicated above.

- 25 Non-peptide mimetics of peptides are discussed further below.

As noted, a peptide according to the present invention and for use in various aspects of the present invention may

- include or consist essentially of a fragment of B7-3 or ICOS as disclosed. Where one or more additional amino acids are included, such amino acids may be from B7-3 or ICOS or may be heterologous or foreign to B7-3 or ICOS. A peptide may also  
5 be included within a larger fusion protein, particularly where the peptide is fused to a non-B7-3 or ICOS (i.e. heterologous or foreign) sequence, such as a polypeptide or protein domain.
- 10 The invention also includes derivatives of the peptides, including the peptide linked to a coupling partner, e.g. an effector molecule, a label, a drug, a toxin and/or a carrier or transport molecule, and/or a targeting molecule such as an antibody or binding fragment thereof or other ligand.
- 15 Techniques for coupling the peptides of the invention to both peptidyl and non-peptidyl coupling partners are well known in the art. In one embodiment, the carrier molecule is a 16 aa peptide sequence derived from the homeodomain of *Antennapedia* (e.g. as sold under the name "Penetratin"), which can be  
20 coupled to a peptide via a terminal Cys residue. The "Penetratin" molecule and its properties are described in WO91/18981.

Peptides may be generated wholly or partly by chemical  
25 synthesis. The compounds of the present invention can be readily prepared according to well-established, standard liquid or, preferably, solid-phase peptide synthesis methods, general descriptions of which are broadly available (see, for



- example, in J.M. Stewart and J.D. Young, Solid Phase Peptide Synthesis, 2nd edition, Pierce Chemical Company, Rockford, Illinois (1984), in M. Bodanzsky and A. Bodanzsky, The Practice of Peptide Synthesis, Springer Verlag, New York
- 5 (1984); and Applied Biosystems 430A Users Manual, ABI Inc., Foster City, California), or they may be prepared in solution, by the liquid phase method or by any combination of solid-phase, liquid phase and solution chemistry, e.g. by first completing the respective peptide portion and then, if
- 10 desired and appropriate, after removal of any protecting groups being present, by introduction of the residue X by reaction of the respective carbonic or sulfonic acid or a reactive derivative thereof.
- 15 Another convenient way of producing a peptidyl molecule according to the present invention (peptide or polypeptide) is to express nucleic acid encoding it, by use of nucleic acid in an expression system.
- 20 Accordingly the present invention also provides in various aspects nucleic acid encoding the peptides of the invention, which may be used for production of the encoded peptide.
- Generally whether encoding for a polypeptide (such as B7-3)
- 25 or peptide in accordance with the present invention, nucleic acid is provided as an isolate, in isolated and/or purified form, or free or substantially free of material with which it is naturally associated, such as free or substantially free

of nucleic acid flanking the gene in the human genome, except possibly one or more regulatory sequence(s) for expression.

Nucleic acid may be wholly or partially synthetic and may include genomic DNA, cDNA or RNA. Where nucleic acid

5 according to the invention includes RNA, reference to the sequence shown should be construed as encompassing reference to the RNA equivalent, with U substituted for T.

Nucleic acid sequences encoding a polypeptide or peptide in  
10 accordance with the present invention can be readily prepared by the skilled person using the information and references contained herein and techniques known in the art (for example, see Sambrook, Fritsch and Maniatis, "Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory  
15 Press (1989), and Ausubel et al., Current Protocols in Molecular Biology, John Wiley and Sons, (1994)), given the nucleic acid sequence and clones available. These techniques include (i) the use of the polymerase chain reaction (PCR) to amplify samples of such nucleic acid, e.g. from genomic  
20 sources, (ii) chemical synthesis, or (iii) preparing cDNA sequences. DNA encoding B7-3 or ICOS fragments may be generated and used in any suitable way known to those of skill in the art, including by taking encoding DNA, identifying suitable restriction enzyme recognition sites  
25 either side of the portion to be expressed, and cutting out said portion from the DNA. The portion may then be operably linked to a suitable promoter in a standard commercially available expression system. Another recombinant approach is

to amplify the relevant portion of the DNA with suitable PCR primers. Modifications to the relevant sequence may be made, e.g. using site directed mutagenesis, to lead to the expression of modified peptide or to take account of codon  
5 preference in the host cells used to express the nucleic acid.

In order to obtain expression of the nucleic acid sequences, the sequences may be incorporated in a vector having one or  
10 more control sequences operably linked to the nucleic acid to control its expression. The vectors may include other sequences such as promoters or enhancers to drive the expression of the inserted nucleic acid, nucleic acid sequences so that the polypeptide or peptide is produced as a  
15 fusion and/or nucleic acid encoding secretion signals so that the polypeptide produced in the host cell is secreted from the cell. Polypeptide can then be obtained by transforming the vectors into host cells in which the vector is functional, culturing the host cells so that the polypeptide  
20 is produced and recovering the polypeptide from the host cells or the surrounding medium. Prokaryotic and eukaryotic cells are used for this purpose in the art, including strains of *E. coli*, yeast, and eukaryotic cells such as COS or CHO cells.

Thus, the present invention also encompasses a method of making a polypeptide or peptide (as disclosed), the method including expression from nucleic acid encoding the

polypeptide or peptide (generally nucleic acid according to the invention). This may conveniently be achieved by growing a host cell in culture, containing such a vector, under appropriate conditions which cause or allow expression of the polypeptide. Polypeptides and peptides may also be expressed in *in vitro* systems, such as reticulocyte lysate.

Systems for cloning and expression of a polypeptide in a variety of different host cells are well known. Suitable host cells include bacteria, eukaryotic cells such as mammalian and yeast, and baculovirus systems. Mammalian cell lines available in the art for expression of a heterologous polypeptide include Chinese hamster ovary cells, HeLa cells, baby hamster kidney cells, COS cells and many others. A common, preferred bacterial host is *E. coli*.

Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator fragments, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. Vectors may be plasmids, viral e.g. 'phage, or phagemid, as appropriate. For further details see, for example, Molecular Cloning: a Laboratory Manual: 2nd edition, Sambrook et al., 1989, Cold Spring Harbor Laboratory Press. Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into

cells and gene expression, and analysis of proteins, are described in detail in Current Protocols in Molecular Biology, Ausubel et al. eds., John Wiley & Sons, 1992.

- 5 Thus, a further aspect of the present invention provides a host cell containing heterologous nucleic acid as disclosed herein.

The nucleic acid of the invention may be integrated into the  
10 genome (e.g. chromosome) of the host cell. Integration may be promoted by inclusion of sequences which promote recombination with the genome, in accordance with standard techniques. The nucleic acid may be on an extra-chromosomal vector within the cell, or otherwise identifiably  
15 heterologous or foreign to the cell.

A still further aspect provides a method which includes introducing the nucleic acid into a host cell. The introduction, which may (particularly for *in vitro*  
20 introduction) be generally referred to without limitation as "transformation", may employ any available technique. For eukaryotic cells, suitable techniques may include calcium phosphate transfection, DEAE-Dextran, electroporation, liposome-mediated transfection and transduction using  
25 retrovirus or other virus, e.g. vaccinia or, for insect cells, baculovirus. For bacterial cells, suitable techniques may include calcium chloride transformation, electroporation

and transfection using bacteriophage. As an alternative, direct injection of the nucleic acid could be employed.

Marker genes such as antibiotic resistance or sensitivity  
5 genes may be used in identifying clones containing nucleic acid of interest, as is well known in the art.

The introduction may be followed by causing or allowing expression from the nucleic acid, e.g. by culturing host  
10 cells (which may include cells actually transformed although more likely the cells will be descendants of the transformed cells) under conditions for expression of the gene, so that the encoded polypeptide (or peptide) is produced. If the polypeptide is expressed coupled to an appropriate signal  
15 leader peptide it may be secreted from the cell into the culture medium. Following production by expression, a polypeptide or peptide may be isolated and/or purified from the host cell and/or culture medium, as the case may be, and subsequently used as desired, e.g. in the formulation of a  
20 composition which may include one or more additional components, such as a pharmaceutical composition which includes one or more pharmaceutically acceptable excipients, vehicles or carriers (e.g. see below).

25 Introduction of nucleic acid encoding a peptidyl molecule according to the present invention may take place *in vivo* by way of gene therapy, to affect, disrupt or interfere with B7-3 function, such as interaction between B7-3 and ICOS.

Thus, a host cell containing nucleic acid according to the present invention, e.g. as a result of introduction of the nucleic acid into the cell or into an ancestor of the cell and/or genetic alteration of the sequence endogenous to the cell or ancestor (which introduction or alteration may take place *in vivo* or *ex vivo*), may be comprised (e.g. in the soma) within an organism which is an animal, particularly a mammal, which may be human or non-human, such as rabbit, guinea pig, rat, mouse or other rodent, cat, dog, pig, sheep, goat, cattle or horse, or which is a bird, such as a chicken. Genetically modified or transgenic animals or birds comprising such a cell are also provided as further aspects of the present invention.

15 This may have a therapeutic aim. (Gene therapy is discussed below.) Also, the presence of a mutant, allele, derivative or variant sequence within cells of an organism, particularly when in place of a homologous endogenous sequence, may allow the organism to be used as a model in testing and/or studying substances which modulate activity of the encoded polypeptide *in vitro* or are otherwise indicated to be of therapeutic potential. Conveniently, however, at least preliminary assays for such substances may be carried out *in vitro*, that is within host cells or in cell-free systems. Where an effect of a test compound is established on cells *in vitro*, those cells or cells of the same or similar type may be grafted into an appropriate host animal for *in vivo* testing.

Suitable screening methods are conventional in the art. They include techniques such as radioimmunosassay, scintillation proximity assay and ELISA methods. For instance, in assaying for substances able to modulate an interaction  
5 between proteins, one of the relevant proteins or a fragment, or an analogue, derivative, variant or functional mimetic thereof, is immobilised whereupon the other is applied in the presence of the agents under test. In a scintillation proximity assay a biotinylated protein fragment may be bound  
10 to streptavidin coated scintillant - impregnated beads (produced by Amersham). Binding of radiolabelled peptide is then measured by determination of radioactivity induced scintillation as the radioactive peptide binds to the immobilized fragment. Agents which intercept this are thus  
15 inhibitors of the interaction.

An example of a scintillation proximity assay for high through-put screening is described by Gorman et al. (1996) J Biol Chem, 271: 6713-6719.

20

In one general aspect, the present invention provides an assay method for a substance with ability to modulate, e.g. disrupt or interfere with interaction between B7-3 and ICOS, the method including:

25 (a) bringing into contact a substance according to the invention including a peptide fragment of B7-3, or a derivative, variant or analogue thereof as disclosed, a substance including the relevant fragment of ICOS or a



variant, derivative or analogue thereof, and a test compound;  
and

(b) determining interaction between said substances.

5 A test compound which disrupts, reduces, interferes with or wholly or partially abolishes interaction between said substances (e.g. including a fragment of either protein), and which may modulate B7-3 and/or ICOS activity, may thus be identified.

10

Agents which increase or potentiate interaction between the two substances may be identified using conditions which, in the absence of a positively-testing agent, prevent the substances interacting.

15

Another general aspect of the present invention provides an assay method for a substance able to interact with the relevant region of B7-3 or ICOS as the case may be, the method including:

- 20 (a) bringing into contact a substance which includes a peptide fragment of B7-3 which interacts with ICOS as disclosed, or which includes a peptide fragment of ICOS which interacts with B7-3, or a variant, derivative or analogue of such peptide fragment, as disclosed, and a test compound; and
- 25 (b) determining interaction between said substance and the test compound.

A test compound found to interact with the relevant portion

of B7-3 may be tested for ability to modulate, e.g. disrupt or interfere with, B7-3 interaction with ICOS and/or ability to affect ICOS activity or other activity mediated by B7-3 as discussed already above, e.g. IL-10 production, TH2 activity, 5 T cell response to antigen (e.g. proliferation, secretion or lymphokines, upregulation of molecules that mediate cell-cell interaction, and/or help for antibody secretion by B cells).

Similarly, a test compound found to interact with the 10 relevant portion of ICOS may be tested for ability to modulate, e.g. disrupt or interfere with, B7-3 activity and/or B7-3 interaction with ICOS and/or ability to affect ICOS activity or other activity mediated by B7-3 and/or ICOS as discussed elsewhere herein, such as IL-10 production, TH2 15 activity, T cell response to antigen (e.g. proliferation, secretion or lymphokines, upregulation of molecules that mediate cell-cell interaction, and/or help for antibody secretion by B cells).

20 Another general aspect of the present invention provides an assay method for a substance able to affect B7-3 activity, the method including:

- (a) bringing into contact B7-3 and a test compound; and
- (b) determining B7-3 activity.

25

B7-3 activity may be determined in the presence and absence of ICOS to allow for an effect of a test compound on activity to be attributed to an effect on interaction between B7-3 and

ICOS, as disclosed.

As noted above, B7-3 interacts with ICOS and may interact with one or more other components of interest, so that  
5 references to ICOS in relation to assays and other aspects of the present invention may be taken to refer to one or more of such other components.

Preliminary assays *in vitro* may be followed by, or run in  
10 parallel with, *in vivo* assays.

Of course, the person skilled in the art will design any appropriate control experiments with which to compare results obtained in test assays.

15 Performance of an assay method according to the present invention may be followed by isolation and/or manufacture and/or use of a compound, substance or molecule which tests positive for ability to modulate B7-3 activity and/or  
20 modulate interaction between B7-3 and ICOS and/or modulate ICOS activity, IL-10 production, TH2 activity or T cell response to antigen (e.g. proliferation, secretion or lymphokines, upregulation of molecules that mediate cell-cell interaction, and/or help for antibody secretion by B cells).

25 The precise format of an assay of the invention may be varied by those of skill in the art using routine skill and

knowledge. For example, interaction between substances may be studied *in vitro* by labelling one with a detectable label and bringing it into contact with the other which has been immobilised on a solid support. Suitable detectable labels, especially for peptidyl substances include <sup>35</sup>S-methionine which may be incorporated into recombinantly produced peptides and polypeptides. Recombinantly produced peptides and polypeptides may also be expressed as a fusion protein containing an epitope which can be labelled with an antibody.

10

The protein which is immobilized on a solid support may be immobilized using an antibody against that protein bound to a solid support or via other technologies which are known *per se*. A preferred approach employs BIACORE and biotinylated

15 beads.

An assay according to the present invention may also take the form of an *in vivo* assay. The *in vivo* assay may be performed upon expression in a cell line such as a yeast strain or mammalian cell line in which the relevant polypeptides or peptides are expressed from one or more vectors introduced into the cell.

Compounds which may be used may be natural or synthetic chemical compounds used in drug screening programmes.

25 Extracts of plants which contain several characterised or uncharacterised components may also be used.

Combinatorial library technology provides an efficient way of testing a potentially vast number of different substances for ability to modulate an interaction with and/or activity of a polypeptide. Such libraries and their use are known in the art, for all manner of natural products, small molecules and peptides, among others. The use of peptide libraries may be preferred in certain circumstances.

Antibodies directed to a site on B7-3 form a further class of putative inhibitor compounds, which also includes antibodies against a site on another polypeptide or peptide which interacts with B7-3 (e.g. ICOS). Candidate inhibitor antibodies may be characterised and their binding regions determined to provide single chain antibodies and fragments thereof which are responsible for disrupting the interaction.

Antibodies may be obtained using techniques which are standard in the art. Methods of producing antibodies include immunising a mammal (e.g. mouse, rat, rabbit, horse, goat, sheep or monkey) with the protein or a fragment thereof.

Antibodies may be obtained from immunised animals using any of a variety of techniques known in the art, and screened, preferably using binding of antibody to antigen of interest. For instance, Western blotting techniques or immunoprecipitation may be used (Armitage et al., (1992) *Nature* 357, 80-82). Isolation of antibodies and/or antibody-producing cells from an animal may be accompanied by a step

of sacrificing the animal.

As an alternative or supplement to immunising a mammal with a peptide, an antibody specific for a protein may be obtained  
5 from a recombinantly produced library of expressed immunoglobulin variable domains, e.g. using lambda bacteriophage or filamentous bacteriophage which display functional immunoglobulin binding domains on their surfaces; for instance see WO92/01047. The library may be naive, that  
10 is constructed from sequences obtained from an organism which has not been immunised with any of the proteins (or fragments), or may be one constructed using sequences obtained from an organism which has been exposed to the antigen of interest.

15

Antibodies according to the present invention may be modified in a number of ways. Indeed the term "antibody" should be construed as covering any binding substance having a binding domain with the required specificity. Thus the invention  
20 covers antibody fragments, derivatives, functional equivalents and homologues of antibodies, including synthetic molecules and molecules whose shape mimicks that of an antibody enabling it to bind an antigen or epitope.

25 Example antibody fragments, capable of binding an antigen or other binding partner are the Fab fragment consisting of the VL, VH, Cl and CH1 domains; the Fd fragment consisting of the VH and CH1 domains; the Fv fragment consisting of the VL and

VH domains of a single arm of an antibody; the dAb fragment which consists of a VH domain; isolated CDR regions and F(ab')<sub>2</sub> fragments, a bivalent fragment including two Fab fragments linked by a disulphide bridge at the hinge region.  
5 Single chain Fv fragments are also included.

A hybridoma producing a monoclonal antibody according to the present invention may be subject to genetic mutation or other changes. It will further be understood by those skilled in  
10 the art that a monoclonal antibody can be subjected to the techniques of recombinant DNA technology to produce other antibodies or chimeric molecules which retain the specificity of the original antibody. Such techniques may involve introducing DNA encoding the immunoglobulin variable region,  
15 or the complementarity determining regions (CDRs), of an antibody to the constant regions, or constant regions plus framework regions, of a different immunoglobulin. See, for instance, EP184187A, GB 2188638A or EP-A-0239400. Cloning and expression of chimeric antibodies are described in EP-A-  
20 0120694 and EP-A-0125023.

Hybridomas capable of producing antibody with desired binding characteristics are within the scope of the present invention, as are host cells, eukaryotic or prokaryotic,  
25 containing nucleic acid encoding antibodies (including antibody fragments) and capable of their expression. The invention also provides methods of production of the antibodies including growing a cell capable of producing the

antibody under conditions in which the antibody is produced, and preferably secreted.

The reactivities of antibodies on a sample may be determined  
5 by any appropriate means. Tagging with individual reporter molecules is one possibility. The reporter molecules may directly or indirectly generate detectable, and preferably measurable, signals. The linkage of reporter molecules may be directly or indirectly, covalently, e.g. via a peptide  
10 bond or non-covalently. Linkage via a peptide bond may be as a result of recombinant expression of a gene fusion encoding antibody and reporter molecule. The mode of determining binding is not a feature of the present invention and those skilled in the art are able to choose a suitable mode  
15 according to their preference and general knowledge.

Antibodies may also be used in purifying and/or isolating a polypeptide or peptide according to the present invention, for instance following production of the polypeptide or  
20 peptide by expression from encoding nucleic acid therefor. Antibodies may be useful in a therapeutic context (which may include prophylaxis) to disrupt B7-3/ICOS interaction with a view to inhibiting their activity. Antibodies can for instance be micro-injected into cells, e.g. at a tumour site,  
25 subject to radio- and/or chemo-therapy (as discussed already above). Antibodies may be employed in accordance with the present invention for other therapeutic and non-therapeutic purposes which are discussed elsewhere herein.



Other candidate inhibitor compounds may be based on modelling the 3-dimensional structure of a polypeptide or peptide fragment and using rational drug design to provide potential inhibitor compounds with particular molecular shape, size and charge characteristics.

A compound found to have the ability to affect B7-3 activity, such as via interaction with ICOS has therapeutic and other potential in a number of contexts, as discussed. For therapeutic treatment such a compound may be used in combination with any other active substance. In such a case, the assay of the invention, when conducted *in vivo*, need not measure the degree of modulation of interaction between, for example, B7-3 and ICOS (or appropriate fragment, variant or derivative thereof) or of modulation of activity caused by the compound being tested. Instead the therapeutic effect may be measured. It may be that such a modified assay is run in parallel with or subsequent to the main assay of the invention in order to confirm that any such effect is as a result of the modulation of B7-3 function, such as inhibition of interaction between B7-3 and ICOS, caused by said inhibitor compound and not merely a general toxic effect.

Thus, an agent identified using one or more primary screens (e.g. in a cell-free system) as having ability to interact with B7-3 and/or ICOS and/or modulate activity of B7-3 may be assessed further using one or more secondary screens. A

secondary screen may involve testing for ability to affect IL-10 production, TH2 activity and so on, as discussed.

Following identification of a substance or agent which  
5 modulates or affects B7-3 activity, the substance or agent may be investigated further. Furthermore, it may be manufactured and/or used in preparation, i.e. manufacture or formulation, of a composition such as a medicament, pharmaceutical composition or drug. These may be  
10 administered to individuals, e.g. for any of the purposes discussed elsewhere herein.

As noted, the agent may be peptidyl, e.g. a peptide which includes a sequence as recited above, or may be a functional  
15 analogue of such a peptide.

As used herein, the expression "functional analogue" relates to peptide variants or organic compounds having the same functional activity as the peptide in question, which may  
20 interfere with the interaction between B7-3 and another cellular component such as ICOS. Examples of such analogues include chemical compounds which are modelled to resemble the three dimensional structure of the relevant domain in the contact area, and in particular the arrangement of the key  
25 amino acid residues as they appear in in the protein.

In a further aspect, the present invention provides the use of the above substances in methods of designing or screening

for mimetics of the substances.

Accordingly, the present invention provides a method of designing mimetics of B7-3 having the biological activity of B7-3, of B7-3 or ICOS binding or inhibition, or the activity of modulating, e.g. inhibiting, B7-3 interaction with another cellular component such as ICOS, said method comprising:

(i) analysing a substance having the biological activity to determine the amino acid residues essential and important for the activity to define a pharmacophore; and,

(ii) modelling the pharmacophore to design and/or screen candidate mimetics having the biological activity.

Suitable modelling techniques are known in the art. This includes the design of so-called "mimetics" which involves the study of the functional interactions fluorogenic oligonucleotide the molecules and the design of compounds which contain functional groups arranged in such a manner that they could reproduced those interactions.

20

The designing of mimetics to a known pharmaceutically active compound is a known approach to the development of pharmaceuticals based on a "lead" compound. This might be desirable where the active compound is difficult or expensive to synthesise or where it is unsuitable for a particular method of administration, e.g. peptides may not be well suited as active agents for oral compositions as they tend to be quickly degraded by proteases in the alimentary canal.

Mimetic design, synthesis and testing may be used to avoid randomly screening large number of molecules for a target property.

- 5 There are several steps commonly taken in the design of a mimetic from a compound having a given target property. Firstly, the particular parts of the compound that are critical and/or important in determining the target property are determined. In the case of a peptide, this can be done
- 10 by systematically varying the amino acid residues in the peptide, e.g. by substituting each residue in turn. These parts or residues constituting the active region of the compound are known as its "pharmacophore".
- 15 Once the pharmacophore has been found, its structure is modelled to according its physical properties, e.g. stereochemistry, bonding, size and/or charge, using data from a range of sources, e.g. spectroscopic techniques, X-ray diffraction data and NMR. Computational analysis, similarity
- 20 mapping (which models the charge and/or volume of a pharmacophore, rather than the bonding between atoms) and other techniques can be used in this modelling process.

In a variant of this approach, the three-dimensional

25 structure of the ligand and its binding partner are modelled. This can be especially useful where the ligand and/or binding partner change conformation on binding, allowing the model to take account of this the design of the mimetic.

A template molecule is then selected onto which chemical groups which mimic the pharmacophore can be grafted. The template molecule and the chemical groups grafted on to it can conveniently be selected so that the mimetic is easy to synthesise, is likely to be pharmacologically acceptable, and does not degrade *in vivo*, while retaining the biological activity of the lead compound. The mimetic or mimetics found by this approach can then be screened to see whether they have the target property, or to what extent they exhibit it. Further optimisation or modification can then be carried out to arrive at one or more final mimetics for *in vivo* or clinical testing.

The mimetic or mimetics found by this approach can then be screened to see whether they have the target property, or to what extent they exhibit it. Further optimisation or modification can then be carried out to arrive at one or more final mimetics for *in vivo* or clinical testing.

Mimetics of this type together with their use in therapy form a further aspect of the invention.

The present invention further provides the use of a peptide which includes a sequence as disclosed, or a derivative, active portion, analogue, variant or mimetic, thereof able to interact with B7-3 or ICOS and/or modulate, e.g. inhibit, interaction between B7-3 and another cellular component such

as ICOS and/or modulate, e.g. inhibit, B7-3 activity, in screening for a substance able to interact with B7-3 and/or other cellular component which interacts with B7-3, such as ICOS, and/or modulate, e.g. inhibit, interaction between B7-3  
5 and such other cellular component, and/or inhibit B7-3 activity.

Generally, such a substance, e.g. inhibitor, according to the present invention is provided in an isolated and/or purified  
10 form, i.e. substantially pure. This may include being in a composition where it represents at least about 90% active ingredient, more preferably at least about 95%, more preferably at least about 98%. Such a composition may, however, include inert carrier materials or other  
15 pharmaceutically and physiologically acceptable excipients. As noted below, a composition according to the present invention may include in addition to an inhibitor compound as disclosed, one or more other molecules of therapeutic use, such as an anti-tumour agent.

20

The present invention extends in various aspects not only to a substance identified as a modulator of B7-3 and ICOS interaction and/or B7-3- mediated activity, property or pathway, such as an activity, property or pathway mediated  
25 via ICOS, in accordance with what is disclosed herein, but also a pharmaceutical composition, medicament, drug or other composition comprising such a substance, a method comprising

- administration of such a composition to a patient, e.g. for a purpose discussed elsewhere herein, which may include preventative treatment, use of such a substance in manufacture of a composition for administration, e.g. for a
- 5 purpose discussed elsewhere herein, and a method of making a pharmaceutical composition comprising admixing such a substance with a pharmaceutically acceptable excipient, vehicle or carrier, and optionally other ingredients.
- 10 A substance according to the present invention such as an inhibitor of B7-3 and ICOS interaction may be provided for use in a method of treatment of the human or animal body by therapy which affects an B7-3-mediated activity in cells, e.g. T cells. Other purposes of a method of treatment
- 15 employing a substance in accordance with the present invention are discussed elsewhere herein.

Thus the invention further provides a method of modulating an B7-3 activity, e.g. for a purpose discussed elsewhere herein,

20 which includes administering an agent which modulates, inhibits or blocks the interaction of B7-3 with ICOS, such a method being useful in treatment where such modulation, inhibition or blocking is desirable, or an agent which increase, potentiates or strengthens interaction of B7-3 with

25 ICOS, useful in treatment where this is desirable.

The invention further provides a method of treatment which includes administering to a patient an agent which interferes

with the interaction of B7-3 and another cellular component, such as ICOS. Exemplary purposes of such treatment are discussed elsewhere herein.

- 5 Whether it is a polypeptide, antibody, peptide, nucleic acid molecule, small molecule, mimetic or other pharmaceutically useful compound according to the present invention that is to be given to an individual, administration is preferably in a "prophylactically effective amount" or a "therapeutically  
10 effective amount" as the case may be, although prophylaxis may be considered therapy), this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated.
- 15 Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors.

A composition may be administered alone or in combination  
20 with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

Pharmaceutical compositions according to the present invention, and for use in accordance with the present  
25 invention, may include, in addition to active ingredient, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not



interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be oral, or by injection, e.g. cutaneous, subcutaneous or intravenous.

5

Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may include a solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

15

For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

25

Liposomes, particularly cationic liposomes, may be used in carrier formulations.

Examples of techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 16th edition, Osol, A. (ed), 1980.

- 5 The agent may be administered in a localised manner to a tumour site or other desired site or may be delivered in a manner in which it targets tumour or other cells.

Targeting therapies may be used to deliver the active agent  
10 more specifically to certain types of cell, by the use of targeting systems such as antibody or cell specific ligands. Targeting may be desirable for a variety of reasons, for example if the agent is unacceptably toxic, or if it would otherwise require too high a dosage, or if it would not  
15 otherwise be able to enter the target cells.

Instead of administering these agents directly, they may be produced in the target cells by expression from an encoding gene introduced into the cells, eg in a viral vector (a  
20 variant of the VDEPT technique - see below). The vector may be targeted to the specific cells to be treated, or it may contain regulatory elements which are switched on more or less selectively by the target cells.

- 25 The agent (e.g. small molecule, mimetic) may be administered in a precursor form, for conversion to the active form by an activating agent produced in, or targeted to, the cells to be treated. This type of approach is sometimes known as ADEPT

or VDEPT, the former involving targeting the activator to the cells by conjugation to a cell-specific antibody, while the latter involves producing the activator, e.g. an enzyme, in a vector by expression from encoding DNA in a viral vector (see  
5 for example, EP-A-415731 and WO 90/07936).

An agent may be administered in a form which is inactive but which is converted to an active form in the body. For instance, the agent may be phosphorylated (e.g. to improve  
10 solubility) with the phosphate being cleaved to provide an active form of the agent in the body.

A composition may be administered alone or in combination with other treatments, either simultaneously or sequentially  
15 dependent upon the condition to be treated, such as *H. pylori* induced peptic ulcers, Crohn's disease, multiple sclerosis, type 1 diabetes mellitus, graft rejection, helminth infections, allergic diseases or any other condition in which a B7-3- or ICOS-mediated effect is desirable.

20

Nucleic acid according to the present invention, encoding a polypeptide or peptide able to modulate, e.g. interfere with, B7-3, and/or induce or modulate activity of other B7-3- or ICOS- mediated cellular pathway or function, may be used in  
25 methods of gene therapy, for instance in treatment of individuals, e.g. with the aim of preventing or curing (wholly or partially) a disorder or for another purpose as

discussed elsewhere herein.

Vectors such as viral vectors have been used in the prior art to introduce nucleic acid into a wide variety of different  
5 target cells. Typically the vectors are exposed to the target cells so that transfection can take place in a sufficient proportion of the cells to provide a useful therapeutic or prophylactic effect from the expression of the desired polypeptide. The transfected nucleic acid may be  
10 permanently incorporated into the genome of each of the targeted cells, providing long lasting effect, or alternatively the treatment may have to be repeated periodically.

15 A variety of vectors, both viral vectors and plasmid vectors, are known in the art, see US Patent No. 5,252,479 and WO 93/07282. In particular, a number of viruses have been used as gene transfer vectors, including papovaviruses, such as SV40, vaccinia virus, herpesviruses, including HSV and EBV,  
20 and retroviruses. Many gene therapy protocols in the prior art have used disabled murine retroviruses.

As an alternative to the use of viral vectors other known methods of introducing nucleic acid into cells includes  
25 electroporation, calcium phosphate co-precipitation, mechanical techniques such as microinjection, transfer mediated by liposomes and direct DNA uptake and receptor-mediated DNA transfer.

Receptor-mediated gene transfer, in which the nucleic acid is linked to a protein ligand via polylysine, with the ligand being specific for a receptor present on the surface of the target cells, is an example of a technique for specifically  
5 targeting nucleic acid to particular cells.

A polypeptide, peptide or other substance able to modulate or interfere with the interaction of the relevant polypeptide, peptide or other substance as disclosed herein, or a nucleic  
10 acid molecule encoding a peptidyl such molecule, may be provided in a kit, e.g. sealed in a suitable container which protects its contents from the external environment. Such a kit may include instructions for use.

15 In further aspects the present invention provides for the provision of purified B7-3. Purified B7-3, for instance about 10% pure, more preferably about 20% pure, more preferably about 30% pure, more preferably about 40% pure, more preferably about 50% pure, more preferably about 60%  
20 pure, more preferably about 70% pure, more preferably about 80% pure, more preferably about 90% pure, more preferably about 95% pure, or substantially pure B7-3 is obtainable, e.g. using antibodies as provided herein.

25 Individuals with defects in *B7-3* may have increased risk of disorders in which IL-10, TH1 or TH2 activity or other B7-3 or ICOS-mediated activity plays a part. Thus, the *B7-3* gene

may be used in diagnostic and prognostic contexts, e.g. testing individuals to see if they are predisposed to such a disorder or testing patients with such a disorder to see if their disease involves B7-3 and/or B7-3 dysfunction.

5

A number of methods are known in the art for analysing biological samples from individuals to determine whether the individual carries a B7-3 allele predisposing them to any particular disorder, such as one or more of the disorders  
10 discussed above. The purpose of such analysis may be used for diagnosis or prognosis, and serve to detect the presence of an existing condition or disorder, to help identify the type of disorder, to assist a physician in determining the severity or likely course of the disorder and/or to optimise  
15 treatment of it. Alternatively, the methods can be used to detect B7-3 alleles that are statistically associated with a susceptibility to a disorder, such as Crohn's disease, multiple sclerosis, type 1 diabetes mellitus and allergic diseases, in the future, identifying individuals who would  
20 benefit from regular screening to provide early diagnosis.

The disclosure herein paves the way for aspects of the present invention to provide the use of materials and methods, such as are disclosed and discussed above, for  
25 establishing the presence or absence in a test sample of an variant form of the gene, in particular an allele or variant specifically associated with a disorder of interest. This

may be for diagnosing a predisposition of an individual to a disorder or disease. It may be for diagnosing a disorder or disease a patient with the disease as being associated with B7-3 mutation. Determination of the presence or absence of wild-type or a particular allele or variant sequence may be used in a pharmacogenomics context, assessing susceptibility of patients or populations of patients to one or more available treatments.

10 Broadly, the methods divide into those screening for the presence of nucleic acid sequences and those that rely on detecting the presence or absence of polypeptide. The methods make use of biological samples from individuals that may or are suspected to contain the nucleic acid sequences or polypeptide. Examples of biological samples include blood, plasma, serum, tissue samples, tumour samples, saliva and urine.

Exemplary approaches for detecting nucleic acid or polypeptides include:

(a) comparing the sequence of nucleic acid in the sample with the B7-3 nucleic acid sequence to determine whether the sample from the patient contains mutations; or,

(b) determining the presence in a sample from a patient of the polypeptide encoded by the B7-3 gene and, if present, determining whether the polypeptide is full length, and/or is mutated, and/or is expressed at the normal level; or,

- (c) using DNA fingerprinting to compare the restriction pattern produced when a restriction enzyme cuts a sample of nucleic acid from the patient with the restriction pattern obtained from normal *B7-3* gene or from known mutations
- 5 thereof; or,
- (d) using a specific binding member capable of binding to a *B7-3* nucleic acid sequence (either a normal sequence or a known mutated sequence), the specific binding member comprising nucleic acid hybridisable with the *B7-3* sequence,
- 10 or substances comprising an antibody domain with specificity for a native or mutated *B7-3* nucleic acid sequence or the polypeptide encoded by it, the specific binding member being labelled so that binding of the specific binding member to its binding partner is detectable; or,
- 15 (e) using PCR involving one or more primers based on normal or mutated *B7-3* gene sequence to screen for normal or mutant *B7-3* gene in a sample from a patient.

In most embodiments for screening for susceptibility alleles,

20 the *B7-3* nucleic acid in the sample will initially be amplified, e.g. using PCR, to increase the amount of the analyte as compared to other sequences present in the sample. This allows the target sequences to be detected with a high degree of sensitivity if they are present in the sample.

25

Tests may be carried out on preparations containing genomic



DNA, cDNA and/or mRNA. Testing cDNA or mRNA has the advantage of the complexity of the nucleic acid being reduced by the absence of intron sequences, but the possible disadvantage of extra time and effort being required in making the preparations. RNA is more difficult to manipulate than DNA because of the wide-spread occurrence of RN'ases.

Nucleic acid in a test sample may be sequenced and the sequence compared with the sequence shown in SEQ ID NO. 1 to determine whether or not a difference is present. If so, the difference can be compared with known susceptibility alleles to determine whether the test nucleic acid contains one or more of the variations indicated, or the difference can be investigated for association with a particular disorder such as cancer.

Since it will not generally be time- or labour-efficient to sequence all nucleic acid in a test sample or even the whole B7-3 gene, a specific amplification reaction such as PCR using one or more pairs of primers may be employed to amplify the region of interest in the nucleic acid, a particular region in which mutations associated with disease susceptibility occur. The amplified nucleic acid may then be sequenced as above, and/or tested in any other way to determine the presence or absence of a particular feature. Nucleic acid for testing may be prepared from nucleic acid removed from cells or in a library using a variety of other techniques such as restriction enzyme digest and

electrophoresis.

Regions of *B7-3* which are of importance to structure and function of the protein include those identified above as  
5 residues 49-56, 61-67, 73-77, 109-120 and 121-134. Allelic variation or mutation in the encoding nucleic acid affecting the encoded amino acid sequence may disrupt folding and function of the protein and thus may be associated with one or more diseases or disorders such as are identified herein.  
10 Accordingly, in diagnostic contexts, the invention provides for determination of a difference in a test sequence from the sequence provided herein at one or more of these regions, at the nucleotide sequence level and/or the amino acid sequence level, by means of any suitable technique at the disposal of  
15 the skilled person as discussed. The relevant portions of the nucleotide sequence encoding these regions are shown in SEQUENCE ID NO. 1 and each represent a further aspect of the present invention, as do probes designed to specifically hybridise with them and primers designed to specifically  
20 amplify these portions.

Nucleic acid may be screened using a variant- or allele-specific probe. Such a probe corresponds in sequence to a region of the *B7-3* gene, or its complement, containing a  
25 sequence alteration known to be associated with disease susceptibility. Under suitably stringent conditions, specific hybridisation of such a probe to test nucleic acid

is indicative of the presence of the sequence alteration in the test nucleic acid. For efficient screening purposes, more than one probe may be used on the same test sample.

- 5 Allele- or variant-specific oligonucleotides may similarly be used in PCR to specifically amplify particular sequences if present in a test sample. Assessment of whether a PCR band contains a gene variant may be carried out in a number of ways familiar to those skilled in the art. The PCR product
- 10 may for instance be treated in a way that enables one to display the mutation or polymorphism on a denaturing polyacrylamide DNA sequencing gel, with specific bands that are linked to the gene variants being selected.
- 15 An alternative or supplement to looking for the presence of variant sequences in a test sample is to look for the presence of the normal sequence, e.g. using a suitably specific oligonucleotide probe or primer.
- 20 Approaches which rely on hybridisation between a probe and test nucleic acid and subsequent detection of a mismatch may be employed. Under appropriate conditions (temperature, pH etc.), an oligonucleotide probe will hybridise with a sequence which is not entirely complementary. The degree of
- 25 base-pairing between the two molecules will be sufficient for them to anneal despite a mis-match. Various approaches are well known in the art for detecting the presence of a mis-match between two annealing nucleic acid molecules.

For instance, RN'ase A cleaves at the site of a mis-match. Cleavage can be detected by electrophoresing test nucleic acid to which the relevant probe or probe has annealed and looking for smaller molecules (i.e. molecules with higher  
5 electrophoretic mobility) than the full length probe/test hybrid. Other approaches rely on the use of enzymes such as resolvases or endonucleases.

Thus, an oligonucleotide probe that has the sequence of a  
10 region of the normal B7-3 gene (either sense or anti-sense strand) in which mutations associated with disease susceptibility are known to occur may be annealed to test nucleic acid and the presence or absence of a mis-match determined. Detection of the presence of a mis-match may  
15 indicate the presence in the test nucleic acid of a mutation associated with disease susceptibility. On the other hand, an oligonucleotide probe that has the sequence of a region of the B7-3 gene including a mutation associated with disease susceptibility may be annealed to test nucleic acid and the  
20 presence or absence of a mis-match determined. The presence of a mis-match may indicate that the nucleic acid in the test sample has the normal sequence. In either case, a battery of probes to different regions of the gene may be employed.

25 The presence of differences in sequence of nucleic acid molecules may be detected by means of restriction enzyme digestion, such as in a method of DNA fingerprinting where

the restriction pattern produced when one or more restriction enzymes are used to cut a sample of nucleic acid is compared with the pattern obtained when a sample containing the normal gene or a variant or allele is digested with the same enzyme  
5 or enzymes.

There are various methods for determining the presence or absence in a test sample of a particular polypeptide, such as the polypeptide with the amino acid sequence shown in SEQ ID  
10 NO. 2 or an amino acid sequence mutant, variant or allele thereof.

A sample may be tested for the presence of a binding partner for a specific binding member such as an antibody (or mixture  
15 of antibodies), specific for one or more particular variants of the polypeptide shown in SEQ ID NO. 2.

A sample may be tested for the presence of a binding partner for a specific binding member such as an antibody (or mixture  
20 of antibodies), specific for the polypeptide shown in SEQ ID NO. 2.

In such cases, the sample may be tested by being contacted with a specific binding member such as an antibody under  
25 appropriate conditions for specific binding, before binding is determined, for instance using a suitable reporter system. Where a panel of antibodies is used, different reporting labels may be employed for each antibody so that binding of

each can be determined.

A specific binding member such as an antibody may be used to isolate and/or purify its binding partner polypeptide from a  
5 test sample, to allow for sequence and/or biochemical  
analysis of the polypeptide to determine whether it has the  
sequence and/or properties of the polypeptide whose sequence  
is shown in SEQ ID NO. 2, or if it is a mutant or variant  
form. Amino acid sequence is routine in the art using  
10 automated sequencing machines.

Nucleic acid according to the present invention, such as a  
full-length coding sequence or oligonucleotide probe or  
primer, may be provided as part of a kit, e.g. in a suitable  
15 container such as a vial in which the contents are protected  
from the external environment. The kit may include  
instructions for use of the nucleic acid, e.g. in PCR and/or  
a method for determining the presence of nucleic acid of  
interest in a test sample. A kit wherein the nucleic acid is  
20 intended for use in PCR may include one or more other  
reagents required for the reaction, such as polymerase,  
nucleosides, buffer solution etc. The nucleic acid may be  
labelled. A kit for use in determining the presence or  
absence of nucleic acid of interest may include one or more  
25 articles and/or reagents for performance of the method, such  
as means for providing the test sample itself, e.g. a swab  
for removing cells from the buccal cavity or a syringe for  
removing a blood sample (such components generally being

sterile). In a further aspect, the present invention provides an apparatus for screening B7-3 nucleic acid, the apparatus comprising storage means including a nucleic acid sequence as set out herein, the stored sequence being used to  
5 compare the sequence of the test nucleic acid to determine the presence of mutations.

Various further aspects and embodiments of the present invention will be apparent to those skilled in the art in  
10 view of the present disclosure. Certain aspects and embodiments of the invention will now be illustrated by way of example and with reference to the sequences discussed already above.

## 15 *EXPERIMENTAL*

As noted, the inventor identified the believed partial KIAA0653 protein sequence (DDBJ/EMBL/GenBank accession number AB014553), to be in fact full length and corresponding to the  
20 partial TU-D sequence of Henry et al. (Immunogenetics (1997) 46, 383-95).

Exercising intuition, the inventor believed this may be a candidate for binding to ICOS ("inducible co-stimulator" -  
25 Hutloff et al. (1999) Nature 397: 263-266), a CD28-related protein.

Human ICOS was shown to bind to TU-D which we hereafter refer to as B7-3, but not bind B7c of Henry et al, *supra*.

### *B7-3 Cloning and Expression*

#### 5 (1) RT-PCR

B7-3 was amplified from 4 $\mu$ g of human B cell RNA using the One Step RT-PCR System (Gibco/BRL).

Conditions used for the RT-PCR were: 0.2 $\mu$ M primers, 0.1mM  
10 magnesium chloride, 200 $\mu$ M dNTPs, (final concentration), 1 $\mu$ l RT/Taq mix, all in 50 $\mu$ l reactions. The PCR cycles used were 1 cycle 50 °C 30 min, 37 cycles 95 °C 15 sec, 55 °C 30 sec, 72 °C 1 min, final 72 °C 10 min.

15 The oligonucleotides used were:

5'oligo: 5'-TAGTAGTCTAGAATCCCCATCCGCTCAAGCAGGCCACC

ATGCGGCTGGGCAGTCCTGGACTG-3'

3'oligo: 5'-CTACTAGTCGACCTCTCTCCGATGTCATTTCTGTCTGG-3'

#### 20 (2) Cloning

The RT-PCR products from two independent PCR reactions were purified using the PCR Purification Kit (Qiagen) and digested using Sal I and Xba I. The digests were cut out of a 1% agarose gel, the DNA purified using the Gel Extraction Kit  
25 (Qiagen) and ligated into pEF BOS/CD4/biotin, which is a vector designed to secrete the protein of interest as a chimera with domains 3 and 4 of CD4, for which



there is a very good antibody (OX-68 - SEROTECH, Kidlington, UK) for purification and immobilization on the BIAcore. Ligations were transformed into TG-1 (CLONTECH) competent cells. Positive colonies were identified by PCR screening  
5 using a 5' pEF BOS oligonucleotide (5'-CAGGGATTTCTTGTCTCCCACG-3') and the 3' B7-3 PCR oligonucleotide indicated above. DNA was extracted and large cultures established from positive colonies from each PCR reaction using a Qiagen Plasmid Purification Kit. Clone  
10 sequences were confirmed by sequencing.

### (3) Transfections

Transient transfections were performed using 293T cells plated in 75 cm flasks. Cells were plated out at a density of  
15  $1 \times 10^5$  cells per ml in DMEM, 10% FCS, 4mM glutamine, 50µg/ml streptomycin, 100µg/ml neomycin, 50 units/ml penicillin, on the day prior to transfection. The following day the medium was replaced with fresh supplemented DMEM and incubated at 37 °C for 3 hours. For generating calcium phosphate  
20 precipitates, mix 1 (20µg of DNA, 248µl 1M CaCl<sub>2</sub> and dH<sub>2</sub>O to 1ml) was added to 1ml of 2 x HBS, dropwise, whilst mixing by bubbling air using a 1ml pipette. The precipitate was allowed to develop at room temperature for 5 mins and added to the flasks containing the 293T cells, which were incubated  
25 overnight at 37 °C. The next day the medium was decanted and replaced with fresh supplemented DMEM. The cells were left for 5-7 days to secrete the protein.

*Binding studies by surface plasmon resonance*

Binding experiments were performed by SPR on a BIAcore instrument upgraded with the BIAcore Upgrade Kit (Pharmacia Biosensor, Uppsala, Sweden). All experiments

5 were performed at 37 °C (except where otherwise indicated) using HBS buffer [25 mM Hepes (pH 7.4), 150 mM NaCl, 3.4 mM EDTA and 0.005% Surfactant P20] supplied by Pharmacia Biosensor. OX-68 was covalently coupled by primary amine groups to the carboxymethylated dextran matrix on a research

10 grade CM5 sensor chip (Pharmacia Biosensor) using the Amine Coupling Kit (Pharmacia Biosensor) as directed by the manufacturers. B7-3/CD4 was passed over the immobilized antibody along with control CD4 chimeras prior to the injection of the soluble ICOS/Fc chimeric protein. Binding

15 was demonstrated as an increase in response units in the flow cell in which only B7-3 and not the control proteins were bound to the OX-68 antibody.

*Synthesis of B7-3 tetramers*

20

Transient expression by the vector known as pEF-bos (Mizushima et al. (1990) Nucleic Acids Res 18: 5322) is driven by the elongation factor 1a promoter and replication of the vector in cells expressing the large T antigen (for

25 example, COS-7 or 293T cells) is driven via the SV40 origin of replication. The vector has a cloning site consisting of an Xba I site.

65

The vector has been modified by insertion of the following fragment:

```

                SmaI
5              .   BamHI
              .   .
              .   .
              .   .
          R P G G S G G G L N D I F E A O K I E
10 CTAGACCCGGGGGATCCGGCGGAGGCCTGAATGACATCTTCGAGGCACAGAAAATCGAGT
    TGGGCCCCCTAGGCCGCCTCCGGACTIONTGTAGAAGCTCCGTGTCTTTTAGCTCA

W H H H H H H
GGCATCACCATCACCATCACTAAG
15 CCGTAGTGGTAGTGGTAGTGAATTCGATC
```

The introduction of this sequence allows in-frame introduction of a polynucleotide encoding the B7-3 extracellular domain and signal peptide of SEQUENCE ID NO. 4, using the BamHI or SmaI sites. The inventor has produced the mature protein of SEQUENCE ID NO. 4 (by expression with the signal sequence being removed following secretion) and shown that it binds ICOS.

25 293T cells are transformed and translation of the construct results in secretion of the extracellular domain fused to the biotinylation signal of the enzyme BirA (underlined above), followed by the 6 x histidine tag allowing purification of

the secreted protein.

The purified protein is biotinylated and tetramers formed by incubation with avidin, as described in O'Callaghan et al  
5 (1999) Anal Biochem 266: 9-15.

All documents mentioned anywhere herein are incorporated by reference.

SEQUENCE ID NO. 1

Human B7-3 encoding nucleotide sequence

ATGCGGCTGGGCAGTCCTGGACTGCTCTTCTGCTCTTCAGCAGCCTTCGAGCTGATACTCAGGAGAAGGAAGT  
CAGAGCGATGGTAGGCAGCGACGTGGAGCTCAGCTGCGCTTGCCCTGAAGGAAGCCGTTTGTATTAAATGATG  
TTTACGTATATTGGCAAACAGTGAGTCGAAAACCGTGGTGACCTACCACATCCCACAGAACAGCTCCTTGGA  
AACGTGGACAGCCGCTACCGGAACCGAGCCCTGATGTACCGGCCGGCATGCTGCGGGGCGACTTCTCCCTGCG  
CTTGTTCAACGTCACCCCCAGGACGAGCAGAAGTTTCACTGCGCTGGTGTGAGCCAAATCCCTGGGATTCCAGG  
AGGTTTTGAGCGTTGAGGTTAACTGTCATGTGGCAGCAAACCTTCAGCGTGCCCGTCGTCAGCGCCCCCACAGC  
CCCTCCCAGGATGAGCTCACCTTCACGTGTACATCCATAAACGGCTACCCCAGGCCAACGTGTACTGGATCAA  
TAAGACGGACAACAGCCTGCTGGACCAGGCTCTGCAGAATGACACCGTCTTCTTGAACATGCGGGGCTTGTATG  
ACGTGGTCAGCGTGCTGAGGATCGCACGGACCCCCAGCGTGAACATTGGCTGCTGCATAGAGAACGTGCTTCTG  
CAGCAGAACCTGACTGTGCGGCAGCCAGACAGGAATGACATCGGAGAGAGAGACAAGATCACAGAGAATCCAGT  
CAGTACCGGCGAGAAAAACGCGGCCACGTGGAGCATCCTGGCTGTCTGTGCTGCTTGTGGTCTGTCGGGTGG  
CCATAGGCTGGGTGTGCAGGGACCGATGCCTCCAACACAGCTATGCAGGTGCCTGGGCTGTGAGTCCGGAGACA  
GAGCTCACTGGTGAGTTTGCCGTGGGAAGCAGCAGGTTCTGGGGGGCCAGGGGAGGCTTGGCTGCCAGCTGTC  
TTTCAGAGTTTCAAAAACTTTCAGAAGGCAAAAGTCCCTTGCTTGAACAACTGTTGTTCTTGGAGACGCAGC  
GAAGCCCTCGATGGTGCGCACGGCATTTCCTGCAGCCTCCCTTGGCATGGGATGGCATCCTGGTGTGCACTTT  
GTCACACTGCGATGGGATTTTCCCAACATGCACAGAAGCAGAGAGACGAGTGCTAGACCCCCGCGCTCCCCAGT  
GCCCAGCCCCGACCAGGGTGTCCAGGGCGGGTCCAGGCACCGGCGCCAGCCCCATGGGGTGTCCGGAGTGGG  
TCCAGGCACCGGCGCCAGCCCCGTGGGGTGTCCAGGGCGGGTCCAGGCACCGGCGCCAGCCCCGTGGGGT  
GTCCAGGGCGGGTCCAGGCACCGGCGCCAGCCCCATGGGGTGTCCGGAGTGGGTCCAGGCACCGGCGCCAG  
CCCCGTGGGGTGTCCAGGGCGGGTCCAGGCACCGGCGCCAGCCCCGTGGGGTGTCTGGAGCGGGTCCGGGC  
ACCGCCAGCTTCTCTGTGTGGCAGCCACTCCTGCAGCTCTCGTTTGCCCTCAGTTCCAGGAGCAACA

SEQUENCE ID NO. 2

## Protein sequence of human B7-3

M R L G S P G L L F L L F S S L R A D T Q E K E V  
R A M V G S D V E L S C A C P E G S R F D L N D V  
Y V Y W Q T S E S K T V V T Y H I P Q N S S L E N  
V D S R Y R N R A L M S P A G M L R G D F S L R L  
F N V T P Q D E Q K F H C L V L S Q S L G F Q E V  
L S V E V T L H V A A N F S V P V V S A P H S P S  
Q D E L T F T C T S I N G Y P R P N V Y W I N K T  
D N S L L D Q A L Q N D T V F L N M R G L Y D V V  
S V L R I A R T P S V N I G C C I E N V L L Q Q N  
L T V G S Q T G N D I G E R D K I T E N P V S T G  
E K N A A T W S I L A V L C L L V V V A V A I G W  
V C R D R C L Q H S Y A G A W A V S P E T E L T G  
E F A V G S S R F W G A Q G R L G C Q L S F R V S  
K N F Q K A K V P C L E Q L L F L E T Q R S P R W  
C A R H F L Q P P L G M G W H P G V H F V T L R W  
D F P N M H R S R E T S A R P P R S P V P S P D Q  
G V Q G G S R H R R P A P M G C P E W V Q A P A P  
S P R G V S R A G P G T G A Q P P W G V Q G G S R  
H R R P A P M G C P E W V Q A P A P S P R G V S R  
A G P G T G A Q P L W G V W S G S G H R Q L L S V  
A A T P A A L V C P S V P G A T

SEQUENCE ID NO. 3

Nucleotide sequence encoding human B7-3 signal sequence and  
extracellular domain

ATGCGGCTGGGCAGTCCTGGACTGCTCTTCCTGCTCTTCAGCAGCCTTCGAGCTGATACTCAGGAGAAGGAAGT  
CAGAGCGATGGTAGGCAGCGACGTGGAGCTCAGCTGCGCTTGCCCTGAAGGAAGCCGTTTTGATTTAAATGATG  
TTTACGTATATTGGCAAACAGTGAGTCGAAAACCGTGGTGACCTACCACATCCCACAGAACAGCTCCTTGAA  
AACGTGGACAGCCGCTACCGGAACCGAGCCCTGATGTACCGGCCGGCATGCTGCGGGGCGACTTCTCCCTGCG  
CTTGTTCAACGTACCCCCCAGGACGAGCAGAAGTTTCACTGCCTGGTGTGAGCCAATCCCTGGGATTCCAGG  
AGGTTTTGAGCGTTGAGGTTACACTGCATGTGGCAGCAAACCTCAGCGTGCCCGTCGTCAGCGCCCCCACAGC  
CCCTCCCAGGATGAGCTCACCTTCACGTGTACATCCATAAACGGCTACCCAGGCCCAACGTGTACTGGATCAA  
TAAGACGGACAACAGCCTGCTGGACCAGGCTCTGCAGAATGACACCGTCTTCTTGAACATGCGGGGCTTGTATG  
ACGTGGTCAGCGTGCTGAGGATCGCACGGACCCCCAGCGTGAACATTGGCTGCTGCATAGAGAACGTGCTTCTG  
CAGCAGAACCTGACTGTCTGGCAGCCAGACAGGAAATGACATCGGAGAGAG

SEQUENCE ID NO. 4

Amino acid sequence of human B7-3 signal sequence and  
extracellular domain.

M R L G S P G L L F L L F S S L R A D T Q E K E V  
R A M V G S D V E L S C A C P E G S R F D L N D V  
Y V Y W Q T S E S K T V V T Y H I P Q N S S L E N  
V D S R Y R N R A L M S P A G M L R G D F S L R L  
F N V T P Q D E Q K F H C L V L S Q S L G F Q E V  
L S V E V T L H V A A N F S V P V V S A P H S P S  
Q D E L T F T C T S I N G Y P R P N V Y W I N K T  
D N S L L D Q A L Q N D T V F L N M R G L Y D V V  
S V L R I A R T P S V N I G C C I E N V L L Q Q N  
L T V G S Q T G N D I G E R

CLAIMS:

1. An isolated B7-3 polypeptide selected from:
  - (i) the human polypeptide of which the amino acid  
5 sequence is shown in SEQ ID NO. 2;
  - (ii) allelic isoforms of (i).
2. An isolated polypeptide including the human polypeptide according to claim 1.  
10
3. An isolated polypeptide according to claim 2 consisting of the amino acid sequence shown in SEQ ID NO. 2.
4. An isolated polypeptide which has an amino acid sequence  
15 which shares at least 80% identity with the polypeptide of claim 3 and which interacts with ICOS.
5. An isolated fragment of a polypeptide according to claim 3, which fragment is at least 5 amino acids in length.  
20
6. An isolated fragment according to claim 5 which is less than about 40 amino acids in length.
7. An isolated fragment according to claim 5 or claim 6  
25 which interacts with ICOS and/or inhibits interaction between B7-3 and ICOS.
8. An isolated fragment according to claim 5 comprising the



B7-3 extracellular domain.

9. An isolated fragment according to claim 8 consisting of the amino acid sequence of SEQ ID NO. 4.

5

10. A B7-3 tetramer comprising a polypeptide according to any one of claims 1 to 4 or a fragment according to claim 8 or claim 9.

10 11. An assay method for obtaining an agent able to interact with a polypeptide or fragment according to any one of claims 1 to 9, the method including:

(a) bringing into contact a substance which includes a said polypeptide or fragment, and a test compound; and

15 (b) determining interaction between said substance and the test compound.

12. An assay method for obtaining an agent with ability to modulate interaction between B7-3 and ICOS, the method

20 including:

(a) bringing into contact a substance which includes a polypeptide according to any one of claims 1 to 3, a polypeptide according to claim 4 able to interact with ICOS, or a fragment according to any one of claims 7 to 9, a

25 substance including ICOS or a portion thereof able to interact with a said polypeptide or fragment, and a test compound; and

(b) determining interaction between said substances.

13. An assay method according to claim 11 or claim 12, the method further including determining ability of a test compound or an agent obtained in the assay to affect B7-3- or ICOS-mediated activity.

5

14. An assay method according to claim 13 which comprises determining IL-10 production by T-cells.

15. An assay method for obtaining an agent able to affect B7-  
10 3 activity, the method including:

(a) bringing into contact a substance which includes a polypeptide according to any one of claims 1 to 3, and a test compound; and

(b) determining B7-3 activity.

15

16. A method comprising:

(a) performing an assay method according to any one of claims 11 to 15 to obtain a said agent;

(b) formulating said agent into a composition which  
20 includes one or more additional components.

17. An agent obtained by a method according to any one of claims 11 to 15.

25 18. An agent according to claim 17 which is a peptide fragment of B7-3 or ICOS.

19. An agent according to claim 17 which is a specific

binding member comprising an antigen-binding domain of an antibody specific for a polypeptide according to claim 1 or specific for ICOS.

- 5 20. An isolated specific binding member comprising an antigen-binding domain of an antibody specific for a polypeptide according to claim 1.
21. Use of a polypeptide or fragment according to any one of  
10 claims 1 to 9 for identifying a substance which interacts with a said polypeptide or fragment.
22. Use of a polypeptide, fragment or tetramer according to any one of claims 1 to 10, an agent according to any one of  
15 claims 17 to 19, or a specific binding member according to claim 20 for modulating an ICOS-mediated activity of T-cells.
23. Use of a polypeptide, fragment or tetramer according to any one of claims 1 to 10, an agent according to any one of  
20 claims 17 to 19, or a specific binding member according to claim 20 in the manufacture of a medicament for modulating an ICOS-mediated activity of T-cells.
24. Use according to claim 22 or claim 23 wherein IL-10  
25 production is inhibited.
25. Use according to claim 22 or claim 23 wherein IL-10 production is stimulated.

26. Use according to claim 22 or claim 23 wherein TH2 activity is inhibited.

27. Use according to claim 22 or claim 23 wherein TH2  
5 activity is stimulated.

28. A method of modulating ICOS-mediated activity of T-cells, the method comprising contacting ICOS on T-cells with a polypeptide, fragment or tetramer according to any one of  
10 claims 1 to 10, an agent according to any one of claims 17 to 19, or a specific binding member according to claim 20.

29. A method according to claim 28 which takes place *in vitro*.

15

30. A method according to claim 28 or claim 29 wherein IL-10 production is inhibited.

31. A method according to claim 28 or claim 29 wherein IL-10  
20 production is stimulated.

32. A method according to claim 28 or claim 29 wherein TH2 activity is inhibited.

25 33. A method according to claim 28 or claim 29 wherein TH2 activity is stimulated.

34. An isolated polynucleotide consisting of a nucleotide sequence encoding a polypeptide according to any one of claims 1 to 4.

5 35. An isolated polynucleotide according to claim 34 of which the sequence encoding said polypeptide is shown in SEQ ID NO. 1.

36. An isolated polynucleotide encoding a fragment according  
10 to any one of claims 5 to 9.

37. An isolated polynucleotide according to claim 36 of which the sequence encoding said fragment is shown in SEQ ID NO. 3.

15 38. An expression vector comprising a polynucleotide according to any one of claims 34 to 37 operably linked to regulatory sequences for expression of said polypeptide or fragment.

20 39. A host cell transformed with an expression vector according to claim 38.

40. A method of making a polypeptide, the method including culturing a host cell according to claim 39 under conditions  
25 for expression of said polypeptide or fragment, and isolating or purifying said polypeptide or fragment.

41. A method according to claim 40 further comprising testing

the polypeptide or fragment for ability to interact with ICOS.

42. A method according to claim 40 or claim 41 wherein the  
5 isolated or purified polypeptide or fragment is formulated  
into a composition comprising one or more additional  
components.

43. A method according to any one of claims 40 to 42 further  
10 comprising bringing the polypeptide or fragment into contact  
with ICOS.

44. A method according to claim 43 which takes place in  
vitro.

15

45. A method which comprises determining in a sample the  
presence or absence of nucleic acid encoding a polypeptide as  
defined in any of claims 1 to 3.

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(54) Title: HUMAN ICOS LIGAND AND APPLICATION THEREOF

(57) Abstract: Human protein termed herein B7-3 encoded by the *B7-3* gene has been cloned and characterised and can be made recombinantly and used. B7-3 protein is a ligand for inducible co-stimulator protein (ICOS). A soluble form of B7-3, for example comprising the extracellular domain shown to bind ICOS, is further provided, as are assay methods for obtaining agents for modulation of the interaction between B7-3 and ICOS.

# INTERNATIONAL SEARCH REPORT

International Application No

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## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K14/705 C12N15/12 C12N15/11 G01N33/68 C07K16/28  
A61K38/17 A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EMBL, STRAND

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	<p>DATABASE EMBL 'Online! Accession number AB014553, 15 July 1998 (1998-07-15) "Homo sapiens mRNA for KIA0653 protein, partial cds." XP002156738 cited in the application the whole document</p>	<p>1,2,4, 17,19, 20,34, 35,38-40</p>
X	<p>-&amp; ISHIKAWA K. ET AL.: "Prediction of the coding sequence of unidentified human genes. X. The complete sequences of 100 new cDNA clones from brain which can code for large proteins in vitro" DNA RESEARCH, vol. 5, 1998, pages 169-176, XP002089186 page 175, line 2 page 176, left-hand column, paragraph 2 --- -/--</p>	<p>1,2,4, 17,19, 20,34, 35, 38-40,45</p>

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents:

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Date of the actual completion of the international search

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# INTERNATIONAL SEARCH REPORT

International Application No  
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL 'Online! Accession number R23544, 23 April 1995 (1995-04-23) XP002156739 the whole document	36
X	-& HENRY J. ET AL.: "Cloning, structural analysis, and mapping of the B30 and B7 multigenic families to the major histocompatibility complex (MHC) and other chromosomal regions" IMMUNOGENETICS, vol. 46, 1997, pages 383-395, XP000971256 cited in the application page 388; figure 6 TU-D page 389, right-hand column, paragraph 5 ---	36
X	WO 99 15553 A (BUNDESREPUBLIK DEUTSCHLAND (DE); ROBERT KOCH INSTITUT (DE); KROCZEK R.) 1 April 1999 (1999-04-01) page 7, line 20-34 page 26 -page 28; claims ---	17-19, 22,23, 28,29
E	WO 00 46240 A (AMGEN INC. (US); YOSHINAGA STEVEN KIYOSHI (US)) 10 August 2000 (2000-08-10)  page 3, line 24 -page 9 page 10; figure 3 page 14; figure 12 page 19, line 33-35 page 49, line 21 -page 52, line 12 page 61, line 14 -page 64, line 13 SEQ ID NO:11,12,13,16-18 page 106 -page 111; claims ---	1,2,4-8, 11-13, 15-23, 25,28, 29,31, 34,36-45
P,X	BRODIE D. ET AL.: "LICOS, a primordial costimulatory ligand?" CURRENT BIOLOGY, vol. 10, no. 6, 10 March 2000 (2000-03-10), pages 333-336, XP000971730 page 333, right-hand column, paragraph 3 -page 334 ---	1,2,4,5, 7,8,10, 11,17, 18,21, 34-36, 38-44
P,X	LING V. ET AL.: "Identification of GL50, a novel B7-like protein that functionally binds to ICOS receptor" THE JOURNAL OF IMMUNOLOGY, vol. 164, no. 4, 15 February 2000 (2000-02-15), pages 1653-1657, XP002156735 the whole document ---	1,2,4,5, 7,8,11, 17,18, 21,34, 36,38-45
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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/GB 00/03079

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HUTLOFF A. ET AL.: "ICOS is an inducible T-cell co-stimulator structurally and functionally related to CD28" NATURE, vol. 397, 21 January 1999 (1999-01-21), pages 263-266, XP002156736 cited in the application ----	
A	ANGELISOVÁ P. ET AL.: "Association of the putative B-lymphocyte surface molecule B7.3 with a protein kinase activity" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 228, 1996, pages 489-493, XP002156737 abstract ----	1,7,8, 12,18
A	WO 95 03408 A (DANA-FARBER CANCER INST (US); REPLIGEN CORP (US); FREEMAN; NADLER ET AL) 2 February 1995 (1995-02-02) abstract page 124 -page 142; claims ----	
T	WANG S. ET AL.: "Costimulation of T cells by B7-H2, a B7-like molecule that binds ICOS" BLOOD, vol. 96, no. 8, 15 October 2000 (2000-10-15), pages 2808-2813, XP002156742 ----	
T	YOSHINAGA S.K. ET AL.: "Characterization of a new human B7-related protein: B7RP-1 is the ligand to the new co-stimulatory protein ICOS" INTERNATIONAL IMMUNOLOGY, vol. 12, no. 10, October 2000 (2000-10), pages 1439-1447, XP000971269 -----	

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

## Continuation of Box I.1

Although claims 22, 24-28 and 30-33 (as far as in vivo methods of treatment of the human/animal body by therapy are concerned, see pages 39-40, 44-51) are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

## Continuation of Box I.2

Claims Nos.: 17 and 22-33 all in part

Present claim 17 relates to an agent defined by reference to the following desirable characteristics or properties: (1) the ability to interact with the polypeptide disclosed in present application, whose amino acid sequence is shown in SEQ ID NO:2, or with fragments thereof; (2) the ability to modulate the interaction between the polypeptide disclosed in present application (indicated as B7-3, whose amino acid sequence is shown in SEQ ID NO:2) and the polypeptide named ICOS (whose amino acid sequence is described in "Nature 1999; 397:263-266 by Hutloff et al.", cited in present application); (3) the ability to affect B7-3- or ICOS-mediated activity.

Present claims 22-33 relate in part to different uses of said agent and to methods involving said agent.

Claim 17 covers all the agents having these characteristics or properties, whereas the application provides support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT for only a very limited number of such agents.

In fact, present application refers in a speculative manner to proteins identified by scanning computer sequence databases (page 6), to non-peptidyl agents (page 7), to antisense molecules (pages 8-9), to ribozymes (page 9), to fragments of the B7-3 polypeptide (pages 14-15), to fragments of ICOS (page 15), to synthetic or chemical compounds (page 34), to anti-B7-3 or anti-ICOS antibodies (page 35), to mimetic compounds (pages 40-43).

However no proteins identified by scanning computer sequence databases, non-peptidyl agents, ribozymes, synthetic or chemical compounds, or mimetic compounds are disclosed in present application.

In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the agent by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to peptide fragments of B7-3 or ICOS, to antisense

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

molecules specific for the gene encoding 87-3 and to binding members comprising an antigen-binding domain of an antibody specific for the polypeptide of present application or specific for ICOS.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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